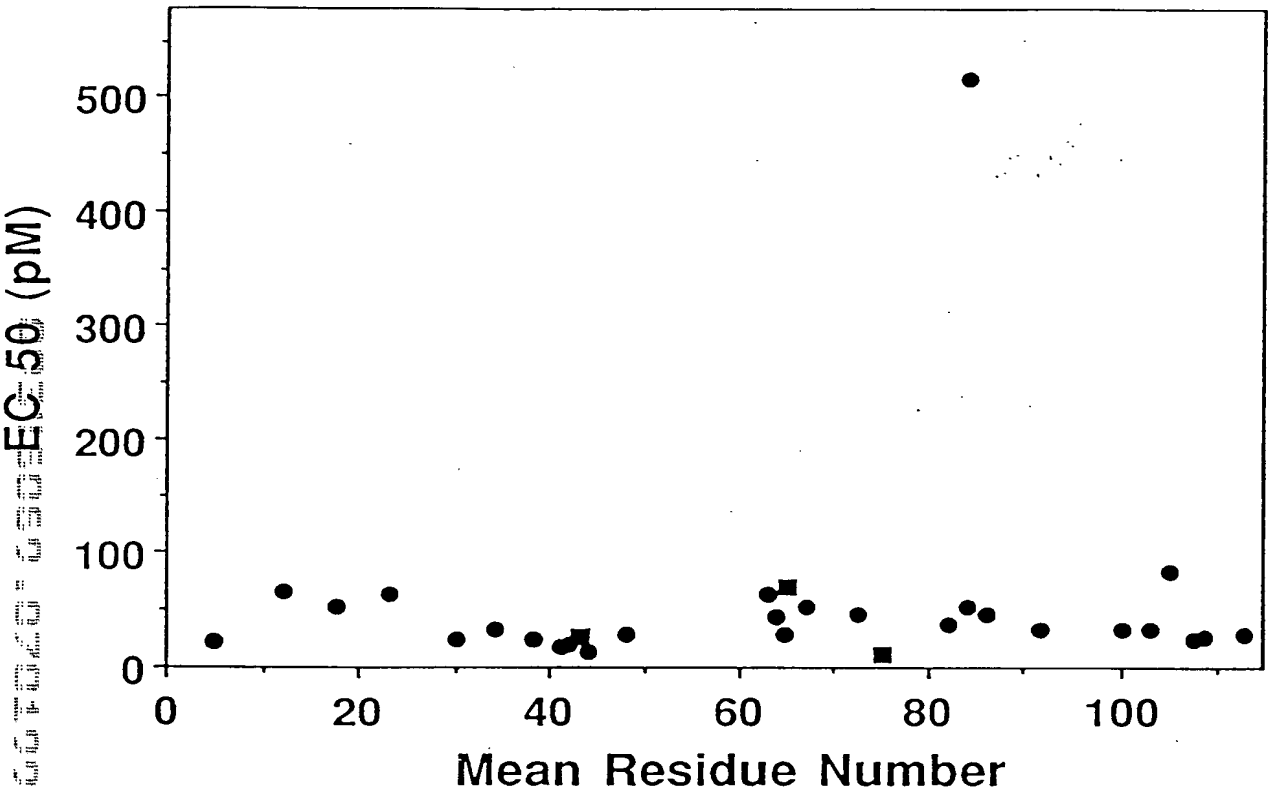


FIG. 21

```
  1  CAGTGTGCTG GCGGCCCGGC GCGAGCCGGC CCGGCCCCGG TCGGGCCTCC
-26

       GAAACC   ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC
                 M   N   F   L   L   S   W   V   H   W   S
               -26                         -20

 90  CTC GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG
-15  L   A   L   L   L   Y   L   H   H   A   K   W   S   Q
                         -10

       GCT|GCA CCC ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC
       A  |A   P   M   A   E   G   G   G   Q   N   H   H
       -1  +1                +5                  +10

171  GAA GTG GTG AAG TTC ATG GAT GTC TAT CAG CGC AGC TAC TGC
 13  E   V   V   K   F   M   D   V   Y   Q   R   S   Y   C
        +15                         +20                 +25

       CAT CCA ATC GAG ACC CTG GTG GAC ATC TTC CAG GAG TAC
       H   P   I   E   T   L   V   D   I   F   Q   E   Y
                  +30                 +35

252  CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA TCC TGT GTG CCC
 40  P   D   E   I   E   Y   I   F   K   P   S   C   V   P
    +40                 +45                 +50

       CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC CTG
       L   M   R   C   G   G   C   C   N   D   E   G   L
          +55                 +60                 +65

333  GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT
 67  E   C   V   P   T   E   E   S   N   I   T   M   Q   I
                +70                 +75                 +80

       ATG CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG
       M   R   I   K   P   H   Q   G   Q   H   I   G   E
                      +85                 +90

414  ATG AGC TTC CTA CAG CAC AAC AAA TGT GAA TGC AGA CCA AAG
 94  M   S   F   L   Q   H   N   K   C   E   C   R   P   K
        +95                 +100                +105

       AAA GAT AGA GCA AGA CAA GAA AAT CCC TGT GGG CCT TGC
       K   D   R   A   R   Q   E   N   P   C   G   P   C
             +110                 +115                +120

495  TCA GAG CGG AGA AAG CAT TTG TTT GTA CAA GAT CCG CAG ACG
121  S   E   R   R   K   H   L   F   V   Q   D   P   Q   T
                   +125                +130

       TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC AAG
       C   K   C   S   C   K   N   T   D   S   R   C   K
    +135                +140                +145
```

FIG. 1A

```
576  GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC
148  A   R   Q   L   E   L   N   E   R   T   C   R   C   D
         +150                    +155                    +160

     AAG CCG AGG CGG TGA GCCGGGCA GGAGGAAGGA GCCTCCCTCA
     K   P   R   R   O
             +165

661  GGGTTTCGGG AACCAGATCT CTCACCAGGA AAGACTGATA CAGAACGATC

     GATACAGAAA CCACGCTGCC GCCACCACAC CATCACCATC GACAGAACAG

761  TCCTTAATCC AGAAACCTGA AATGAAGGAA GAGGAGACTC TGCGCAGAGC

     ACTTTGGGTC CGGAGGGCGA GACTCCGGCG GAAGCATTCC CGGGCGGGTG

861  ACCCAGCACG GTCCCTCTTG GAATTGGATT CGCCATTTTA TTTTTCTTGC

     TGCTAAATCA CCGAGCCCGG AAGATTAGAG AGTTTTATTT CTGGGATTCC

961  TGTAGACACA CCGCGGCCGC CAGCACACTG
```

FIG. 1B

Plasmin releases the heparin-binding domains of VEGF165

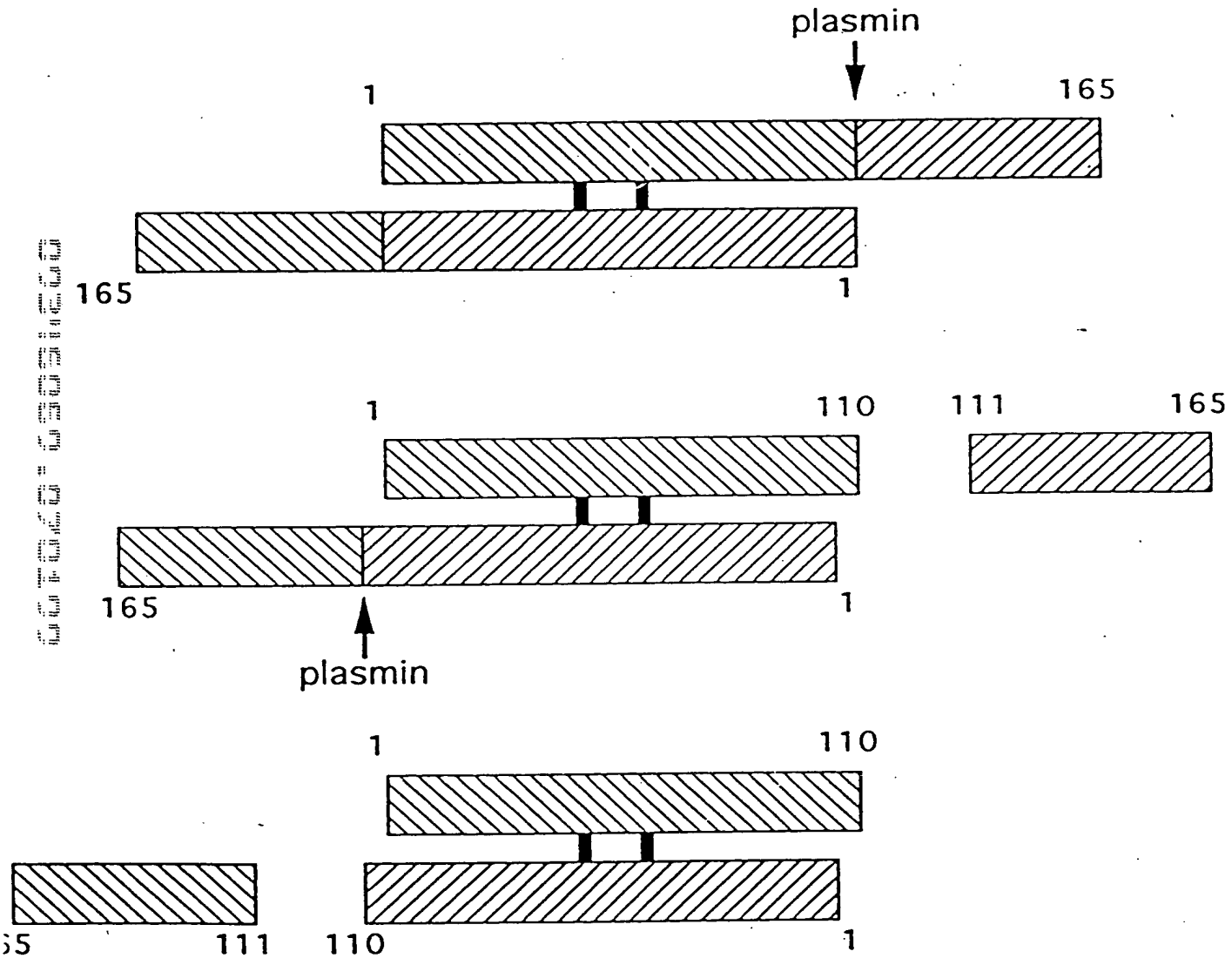


FIG. 2

VEGF displays separate and distinct receptor binding sites for KDR and FLT

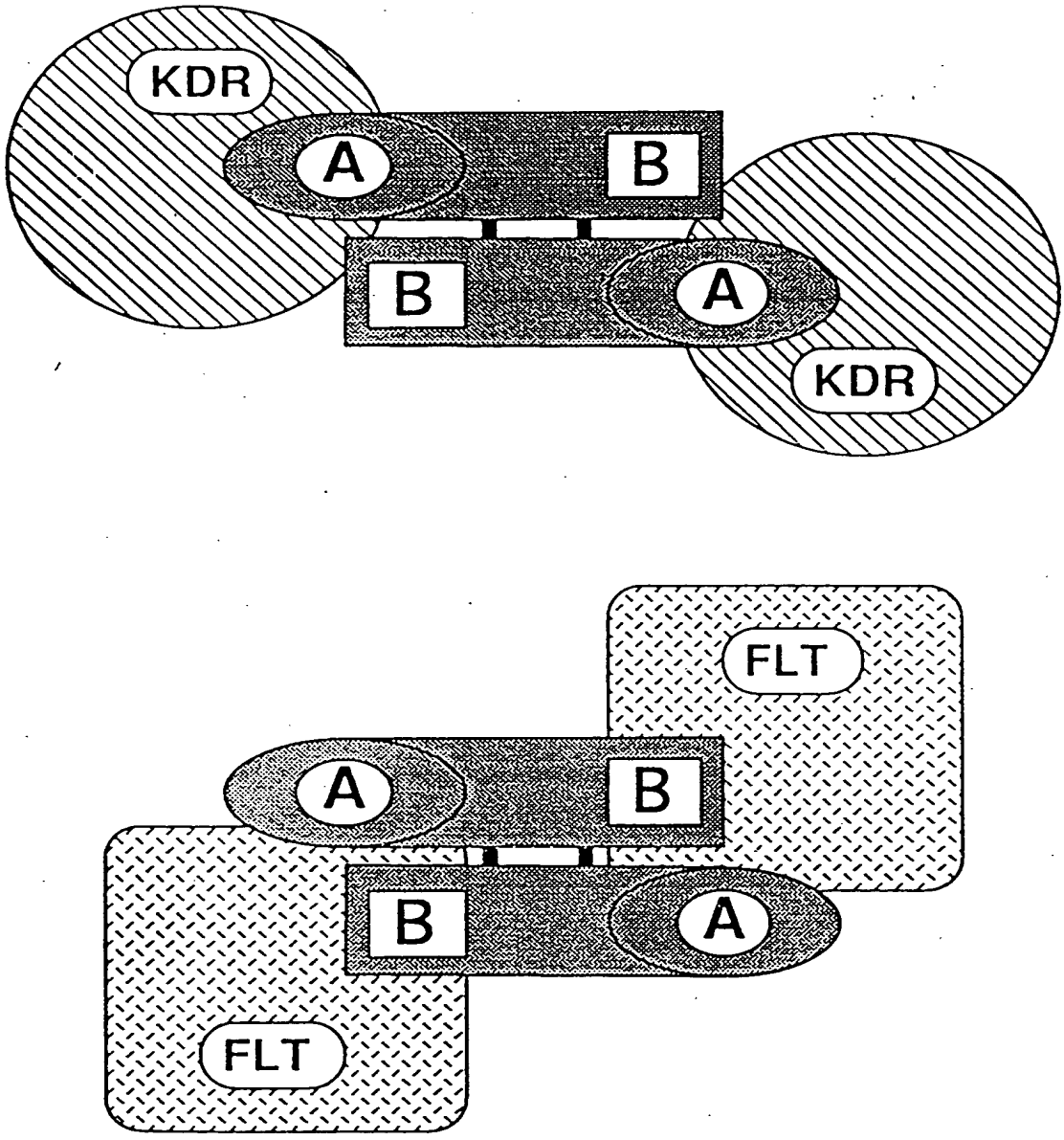


FIG. 3

KDR receptor binding is mediated by the (1-110) dimer of VEGF

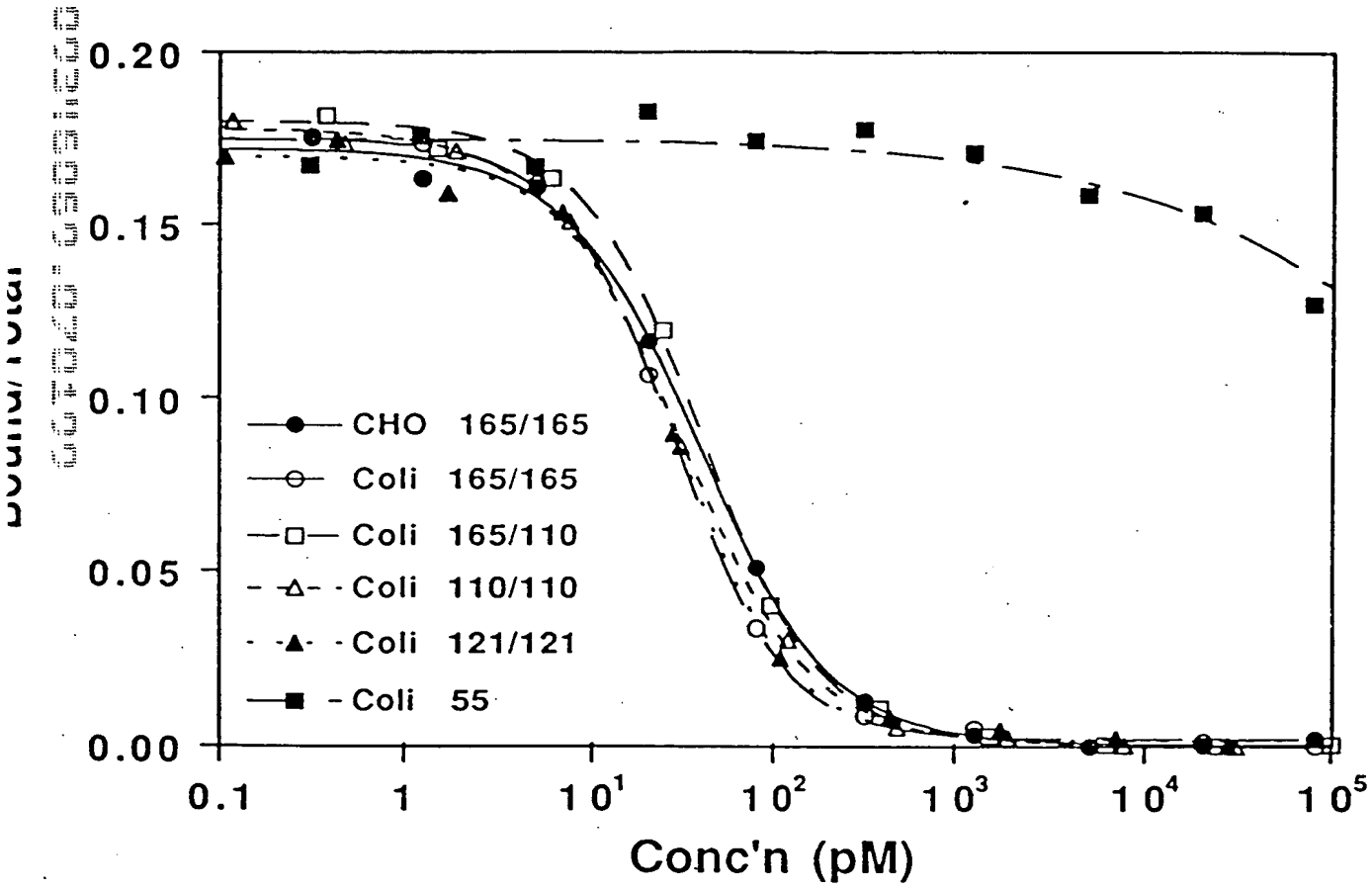


FIG. 4

Charged-to-Alanine Scan Mutations in VEGF

Loci	Mutation
5	E5A
12	H11A, H12A, E13A
17.5	K16A, D19T
23	R23A
27	H27A
28.5	H27A, E30A
30	E30A
34	D34A
36	D34A, E38A
38	E38A
41	D41A
42	E42A
42.3	D41A, E42A, E44A
44	E44A
48	K48A
56	R56A
63	D63A

Loci	Mutation
64	E64A
64.7	D63A, E64A, E67A
67	E67A
72.5	E72A, E73A
82	R82A
84	K84A
84	R82A, K84A, H86A
86	H86A
91.5	H90A, E93A
100	H99A, K101A
103	E103A
105	R105A
107.5	K107A, K108A
108.5	KKDR(107-110)AAAA
109.5	D109A, R110A
113	R112A, E114A

FIG. 5

KDR Binding is primarily mediated by R82, K84, H86

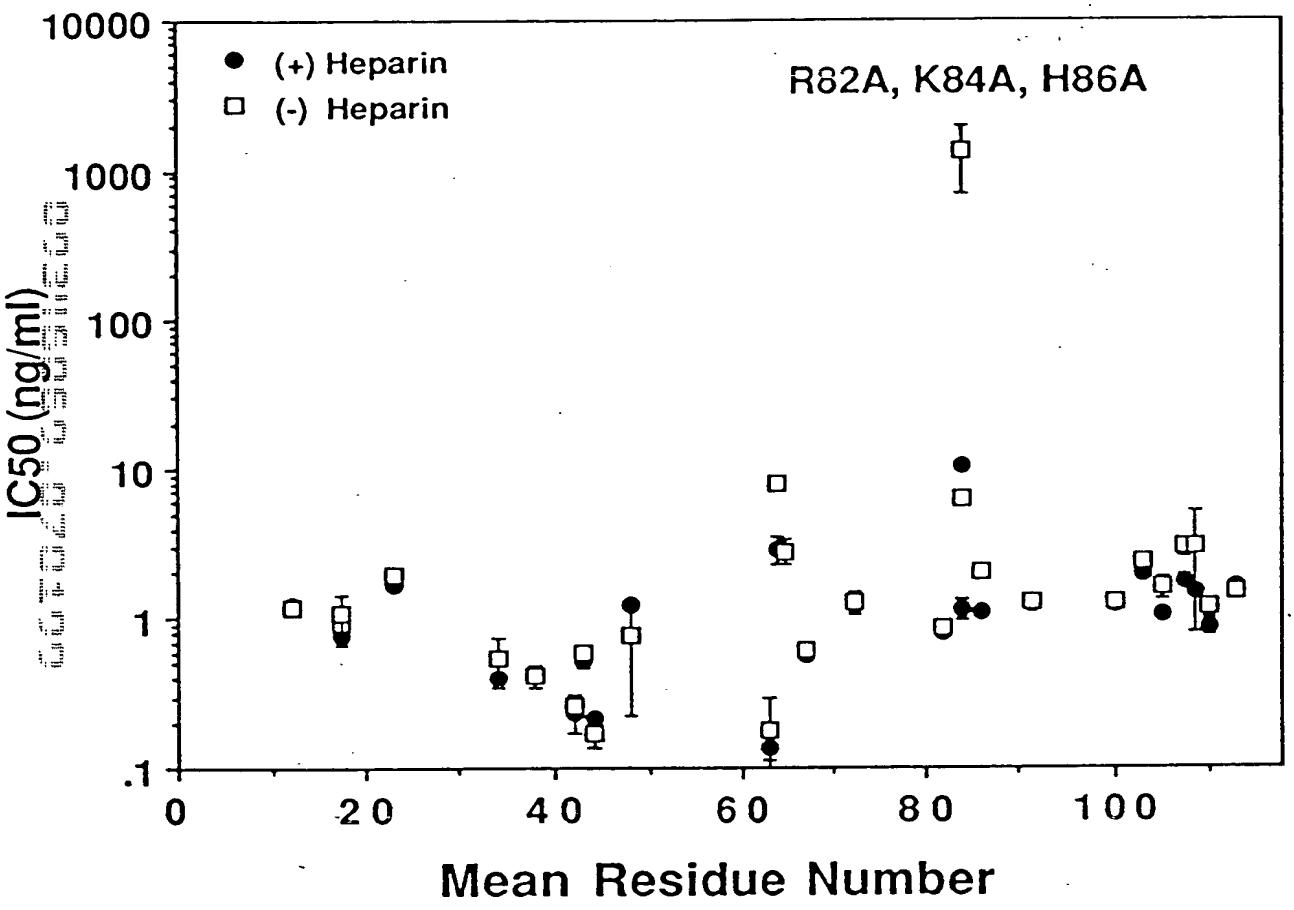


FIG. 6

FLT-1 Binding is mediated by D63, E64, E67

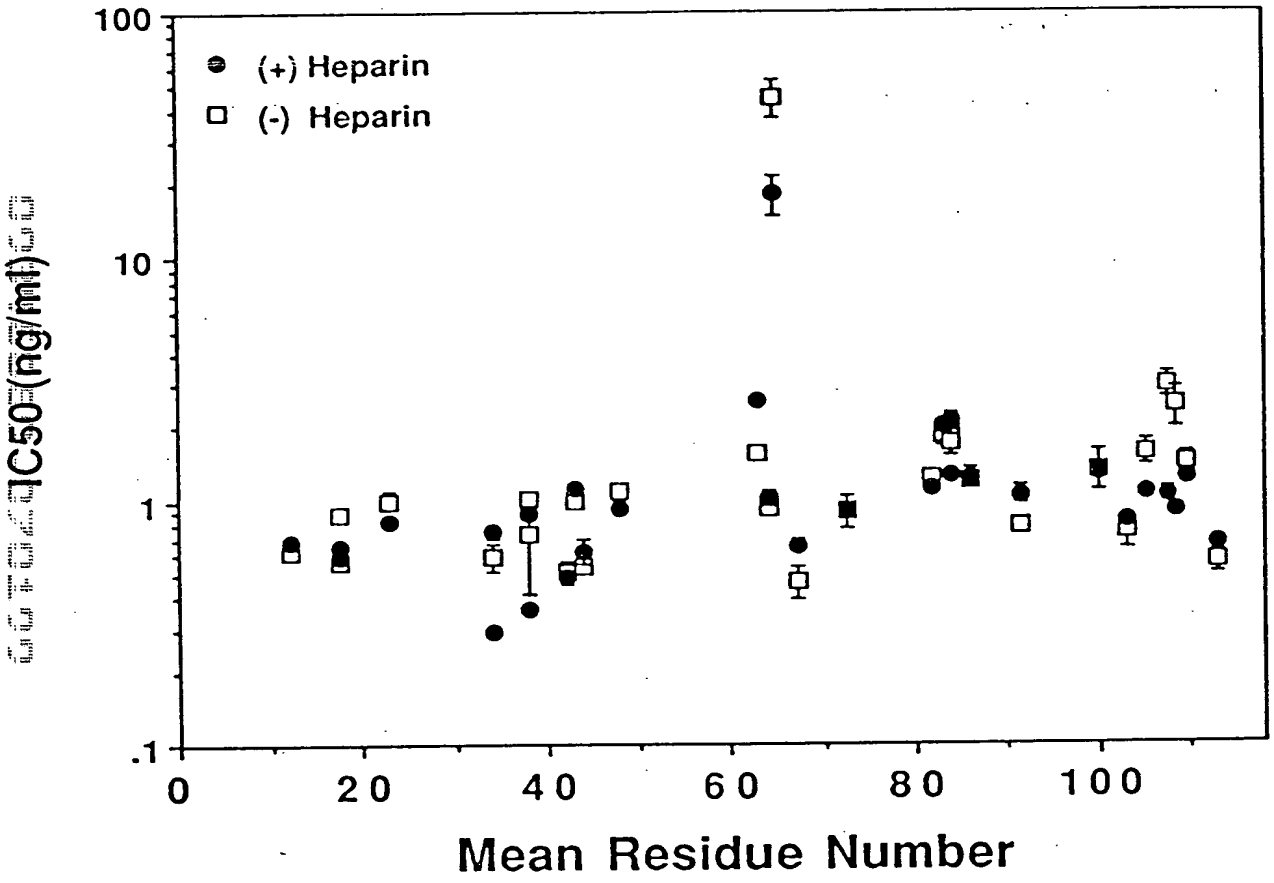


FIG. 7

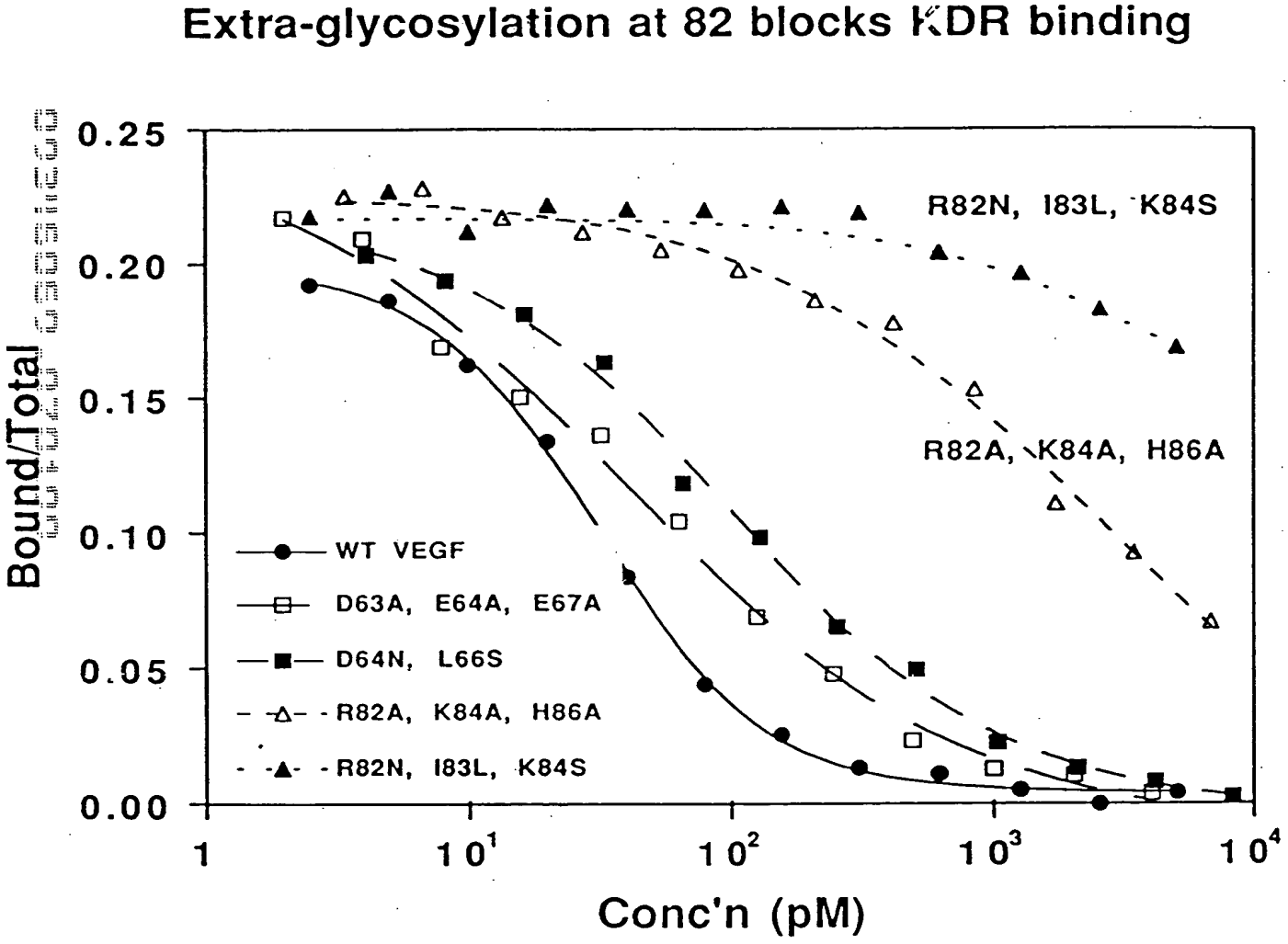

Extra-glycosylation at 82 blocks KDR binding
Bound/Total
0.25
0.20
0.15
0.10
0.05
0.00
1
10^1
10^2
10^3
10^4
Conc'n (pM)
R82N, I83L, K84S
R82A, K84A, H86A
WT VEGF
D63A, E64A, E67A
D64N, L66S
R82A, K84A, H86A
R82N, I83L, K84S

FIG. 8

Mutations in 82-86 site block KDR binding

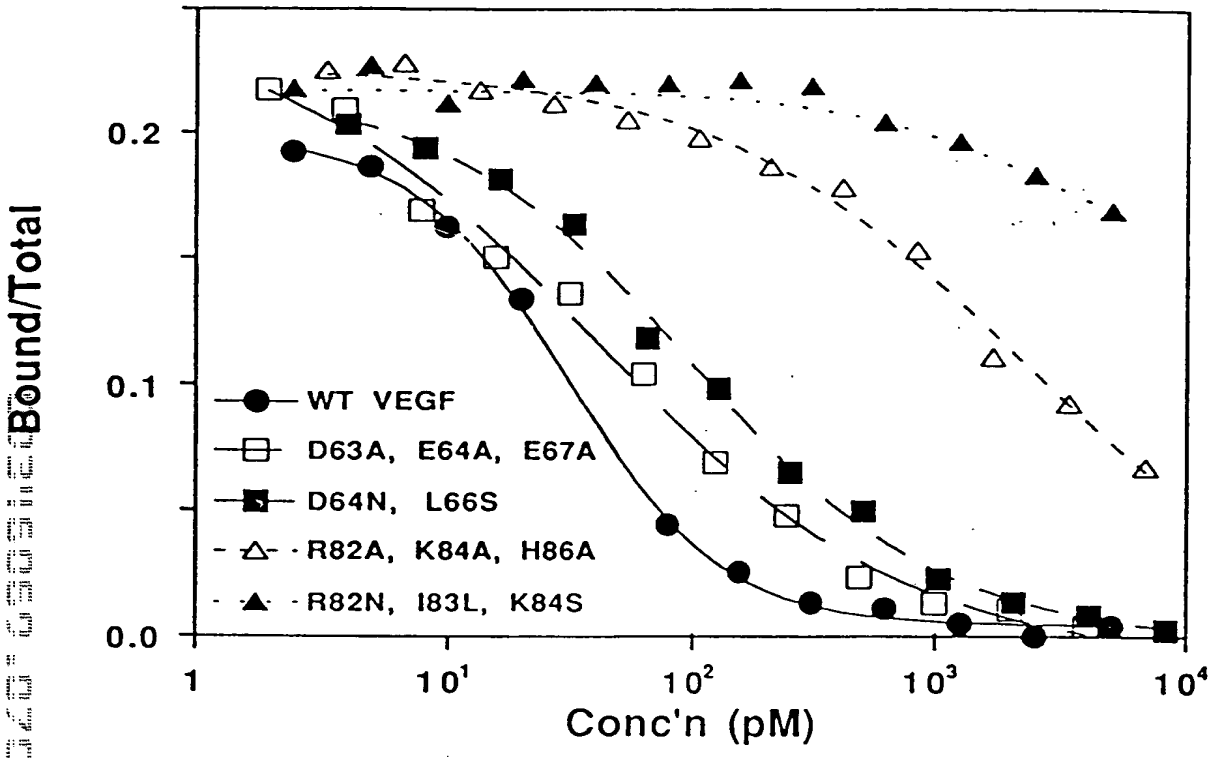


FIG. 9A

Mutations in 63-67 site block FLT binding

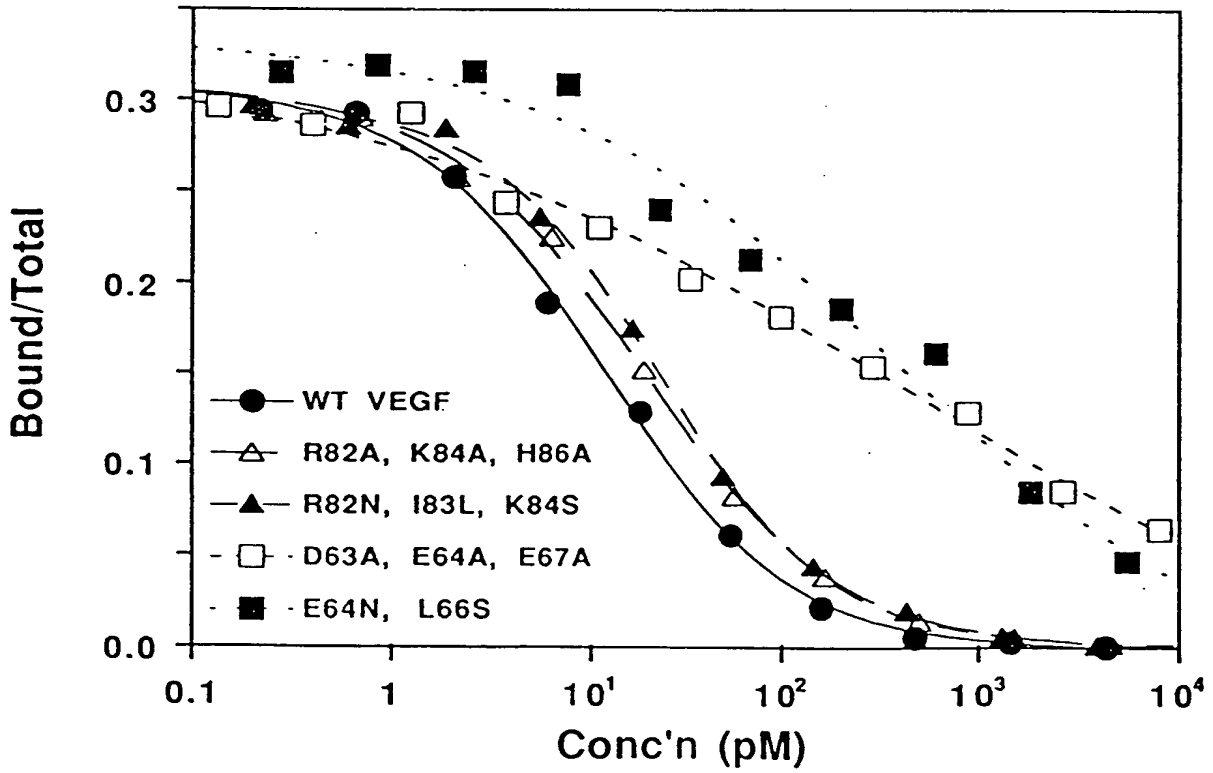


FIG. 9B

Multiple mutations have synergistic effect with KDR: K84A is a potent single alanine substitution

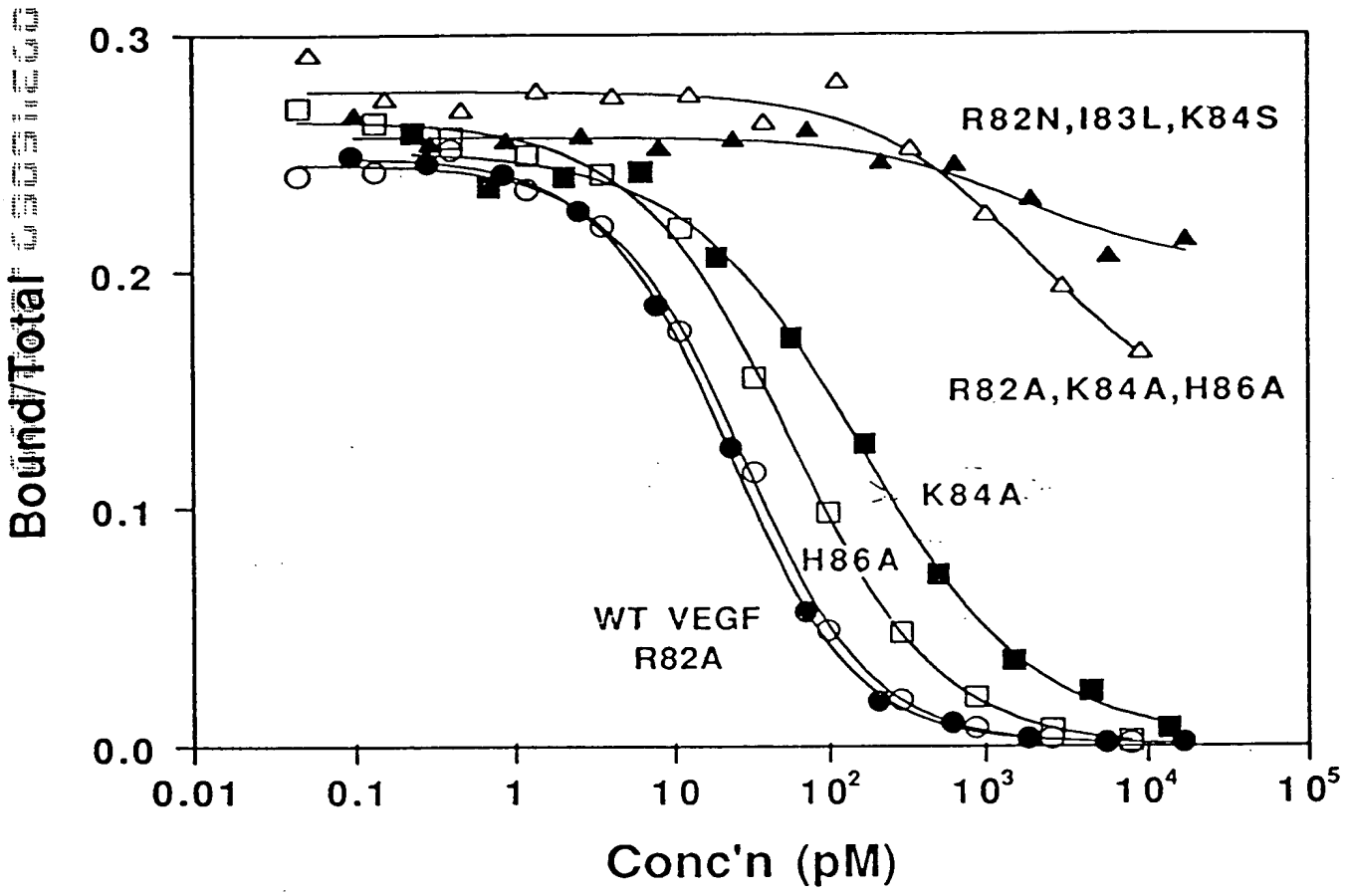


FIG. 10

VEGF Mutants with Decreased KDR Receptor Binding are Weak Endothelial Cell Mitogens

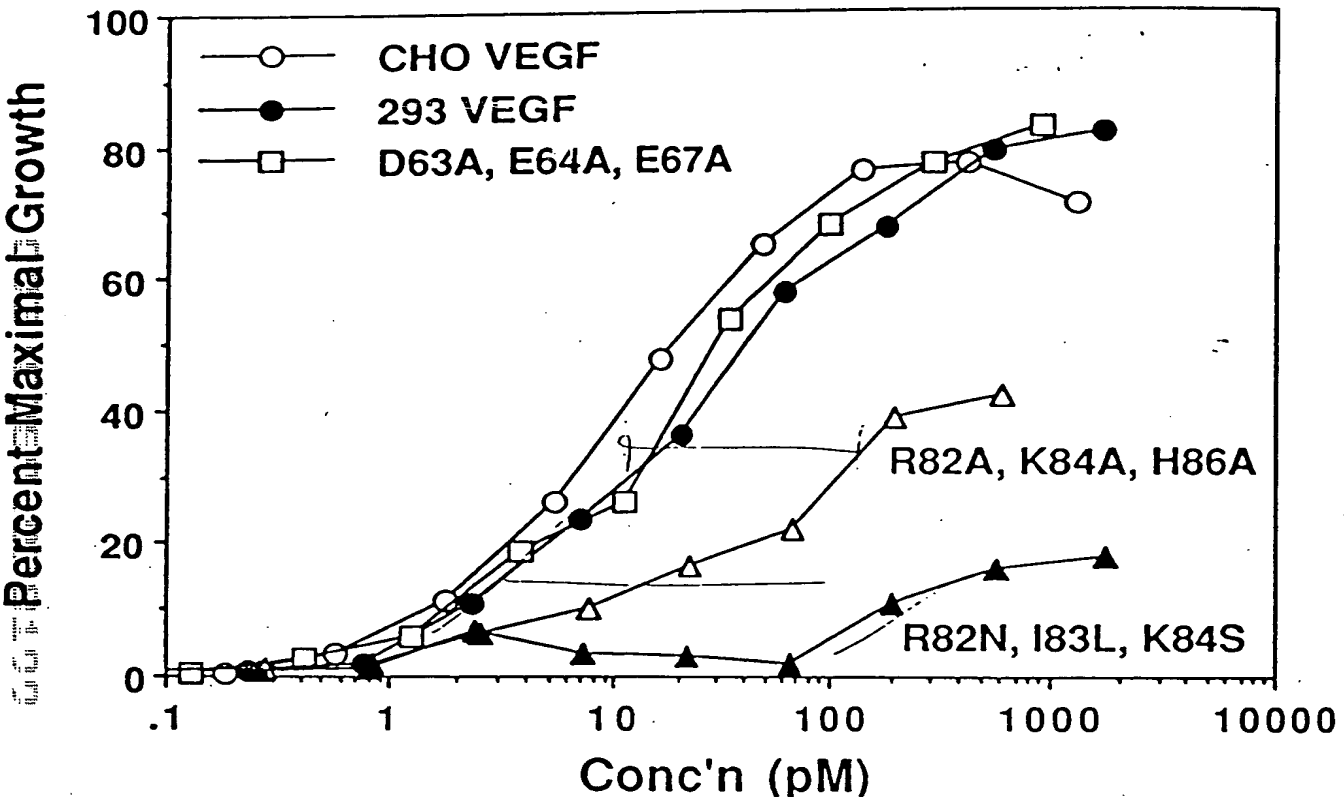
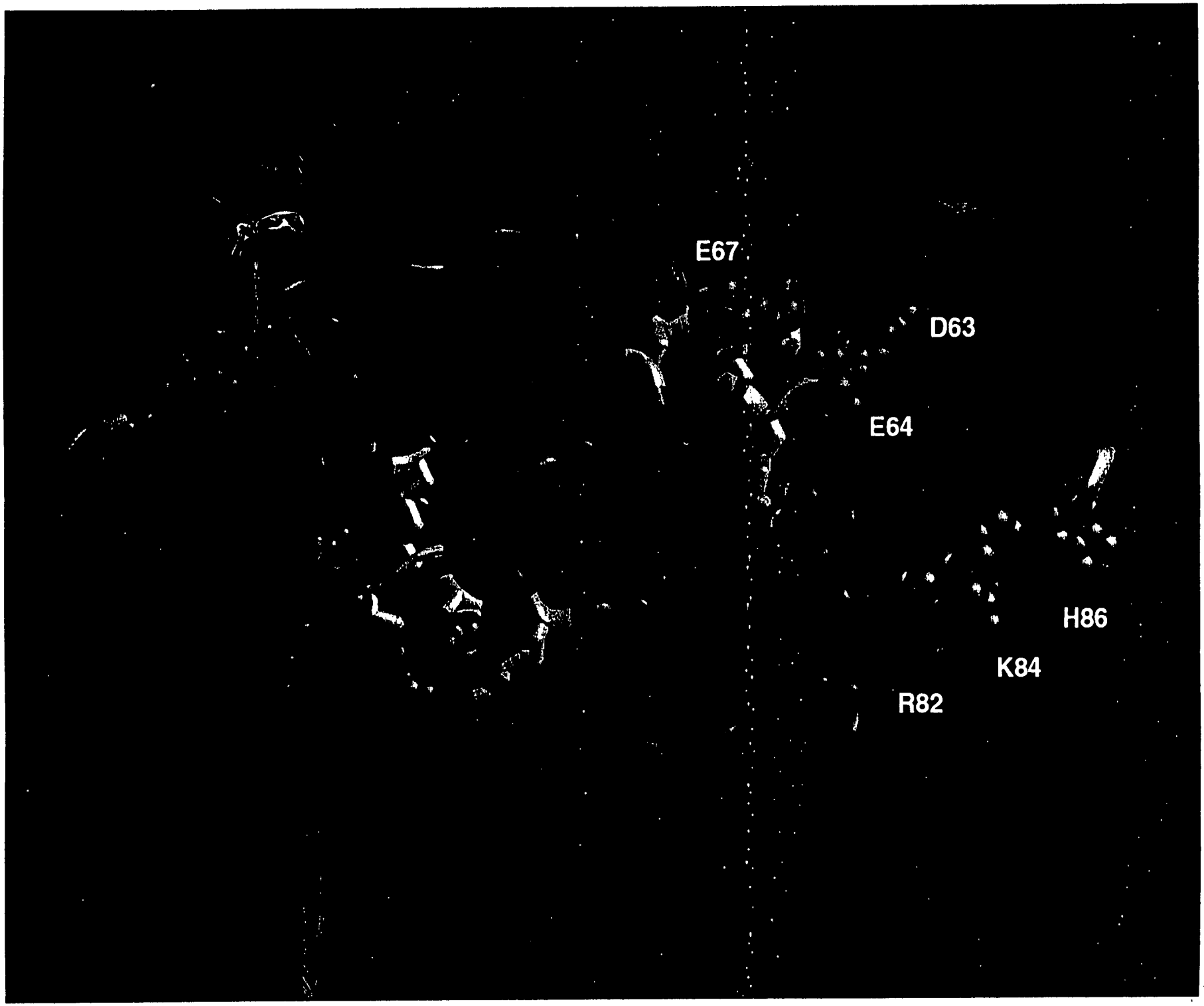


FIG. 11


E67
D63
E64
H86
K84
R82

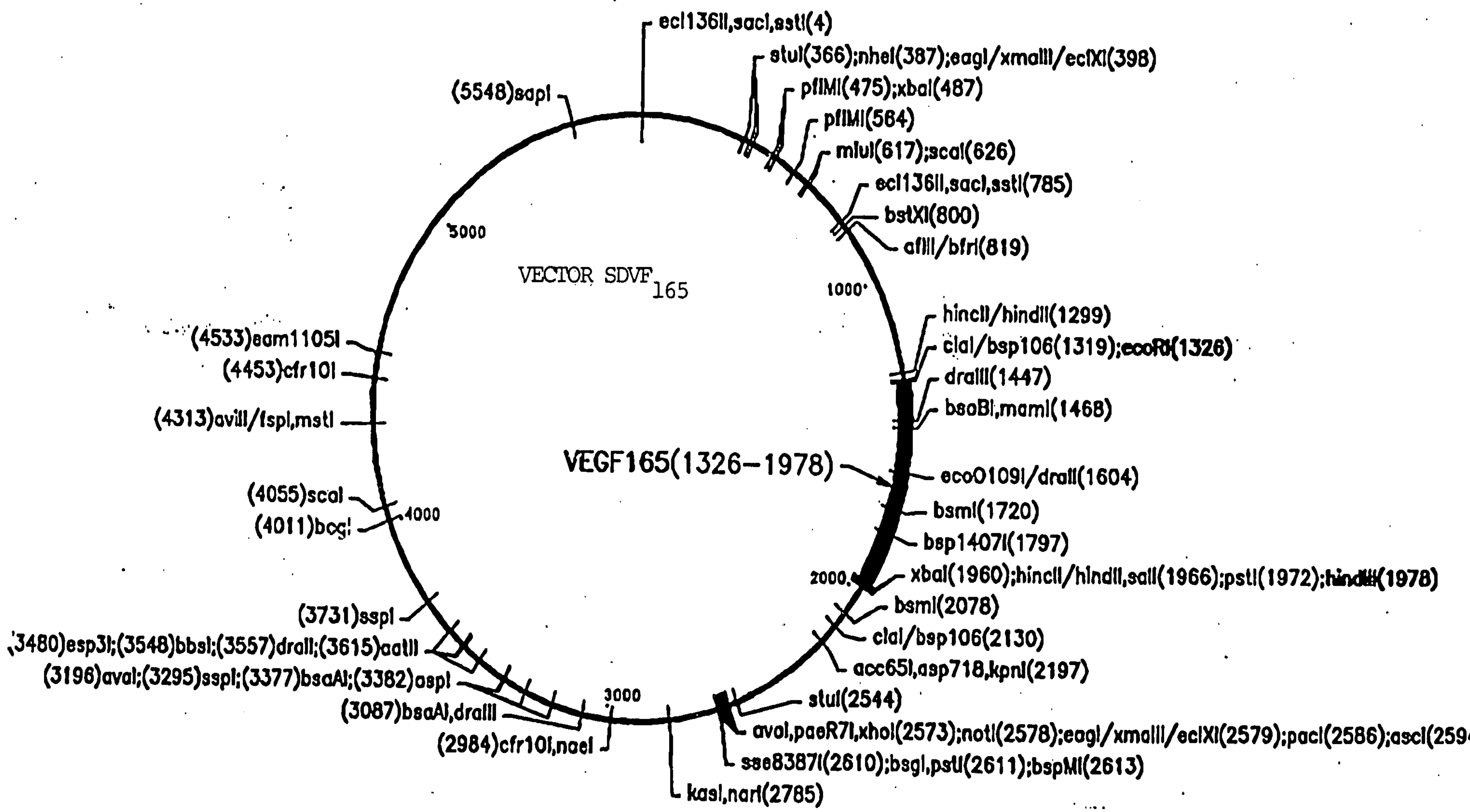


FIGURE 13

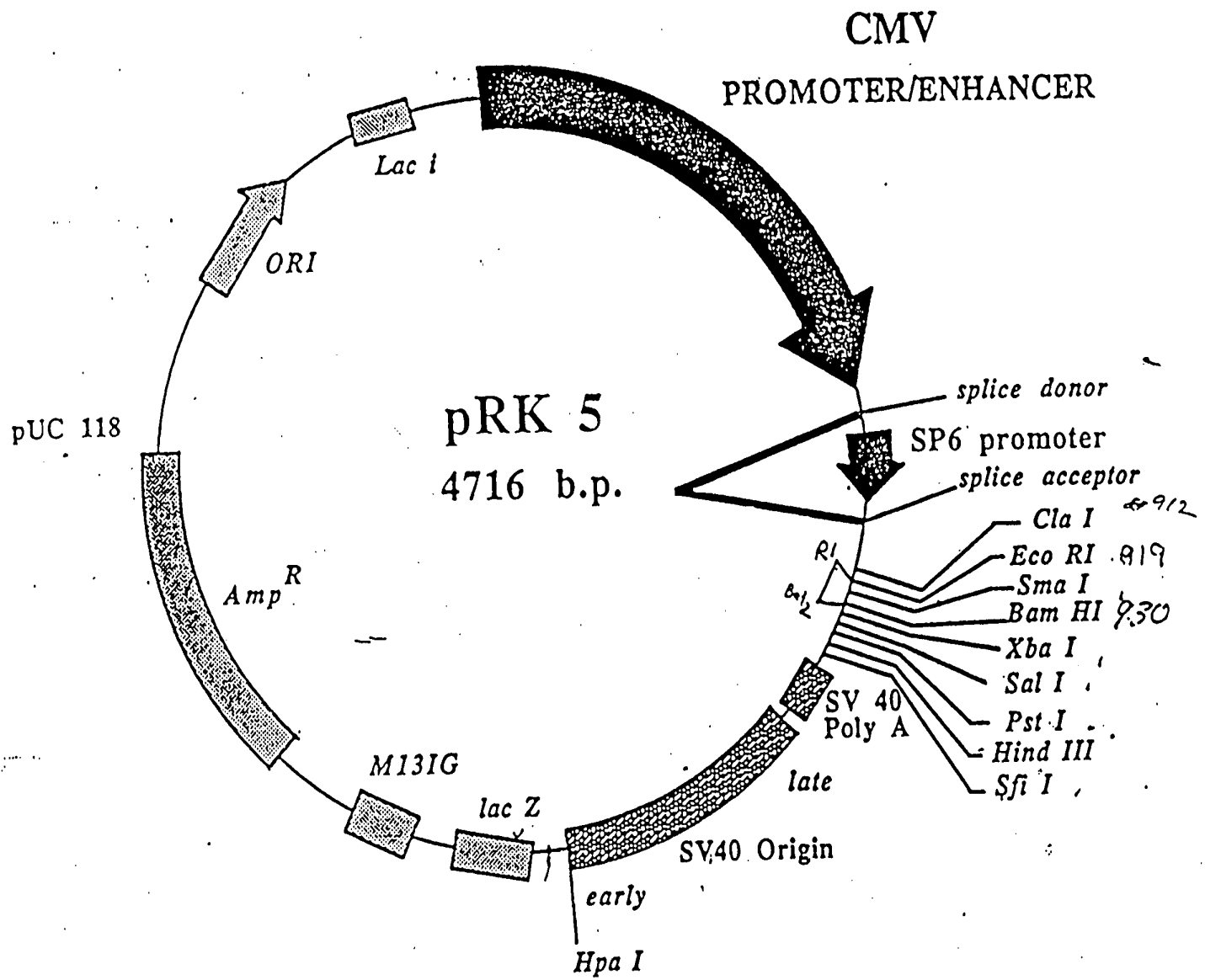

CMV
PROMOTER/ENHANCER
Lac i
ORI
pUC 118
pRK 5
4716 b.p.
splice donor
SP6 promoter
splice acceptor
Cla I
Eco RI
Sma I
Bam HI
Xba I
Sal I
Pst I
Hind III
Sfi I
SV 40
Poly A
late
SV40 Origin
early
Hpa I
M13IG
lac Z
Amp R

FIG. 14

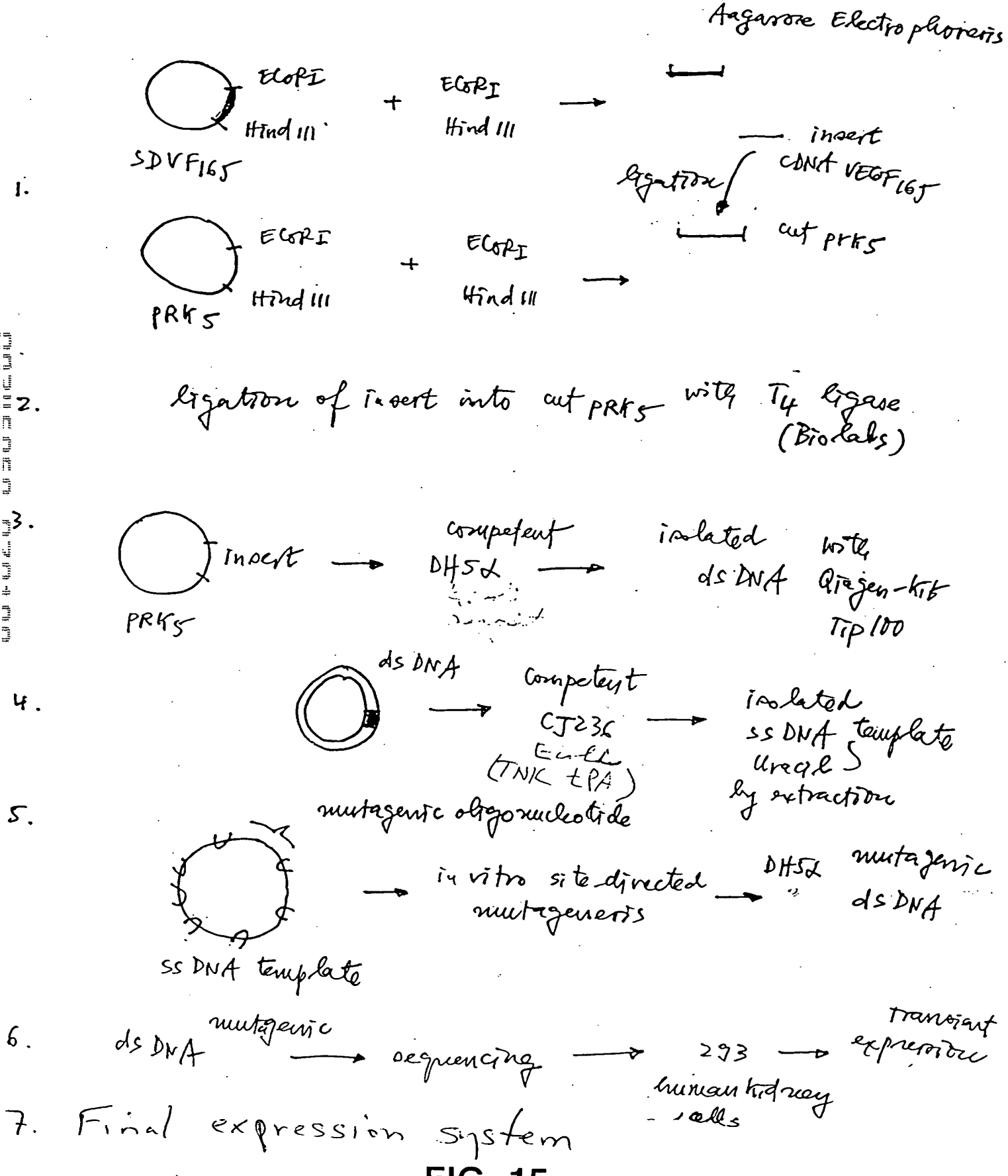

Agarose Electrophoresis
SDVF165
EcoRI
Hind III
EcoRI
Hind III
insert
cDNA VEGF165
ligation
cut PRK5
1.
PRK5
EcoRI
Hind III
EcoRI
Hind III
2. ligation of insert into cut PRK5 with T4 ligase (Biolabs)
3.
insert
PRK5
competent
DH5α
isolated
ds DNA
with
Qiagen-kit
Tip 100
4.
ds DNA
competent
CJ236
(TNK tPA)
isolated
ss DNA template
Ureg 5
by extraction
5.
mutagenic oligonucleotide
ss DNA template
in vitro site-directed
mutagenesis
DH5α
mutagenic
ds DNA
6.
ds DNA
mutagenic
sequencing
293
human kidney
cells
transient
expression
7. Final expression system

FIG. 15

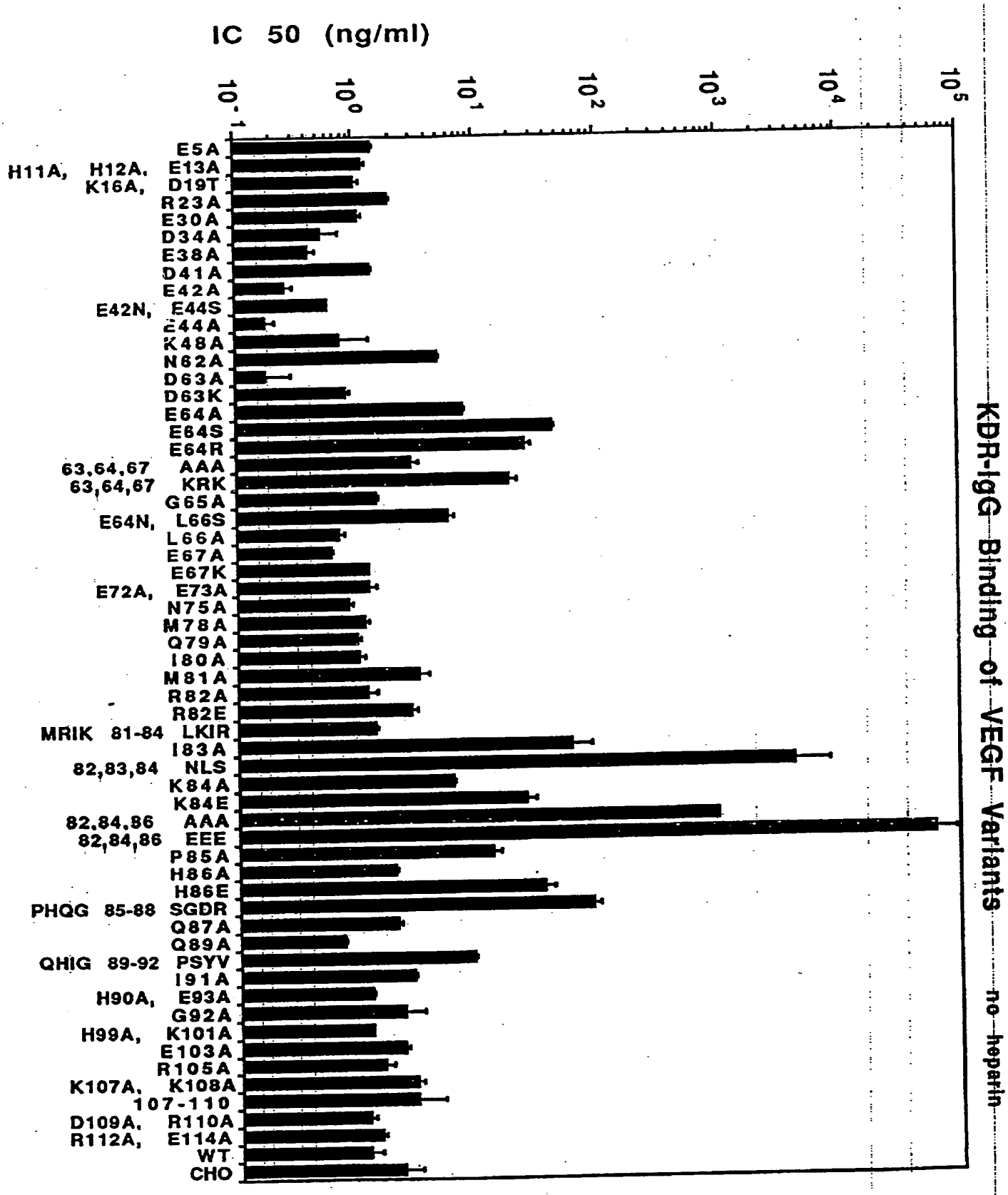

IC 50 (ng/ml)
10⁻¹
10⁰
10¹
10²
10³
10⁴
10⁵
KDR-IgG Binding of VEGF Variants
no heparin
E5A
H11A, H12A, E13A
K16A, D19T
R23A
E30A
D34A
E38A
D41A
E42A
E42N, E44S
E44A
K48A
N62A
D63A
D63K
E64A
E64S
E64R
63,64,67 AAA
63,64,67 KRK
G65A
E64N, L66S
L66A
E67A
E67K
E72A, E73A
N75A
M78A
Q79A
I80A
M81A
R82A
R82E
MRIK 81-84 LKIR
I83A
82,83,84 NLS
K84A
K84E
82,84,86 AAA
82,84,86 EEE
P85A
H86A
H86E
PHQG 85-88 SGDR
Q87A
Q89A
QHIG 89-92 PSYV
I91A
H90A, E93A
G92A
H99A, K101A
E103A
R105A
K107A, K108A
107-110
D109A, R110A
R112A, E114A
WT
CHO

FIG. 16

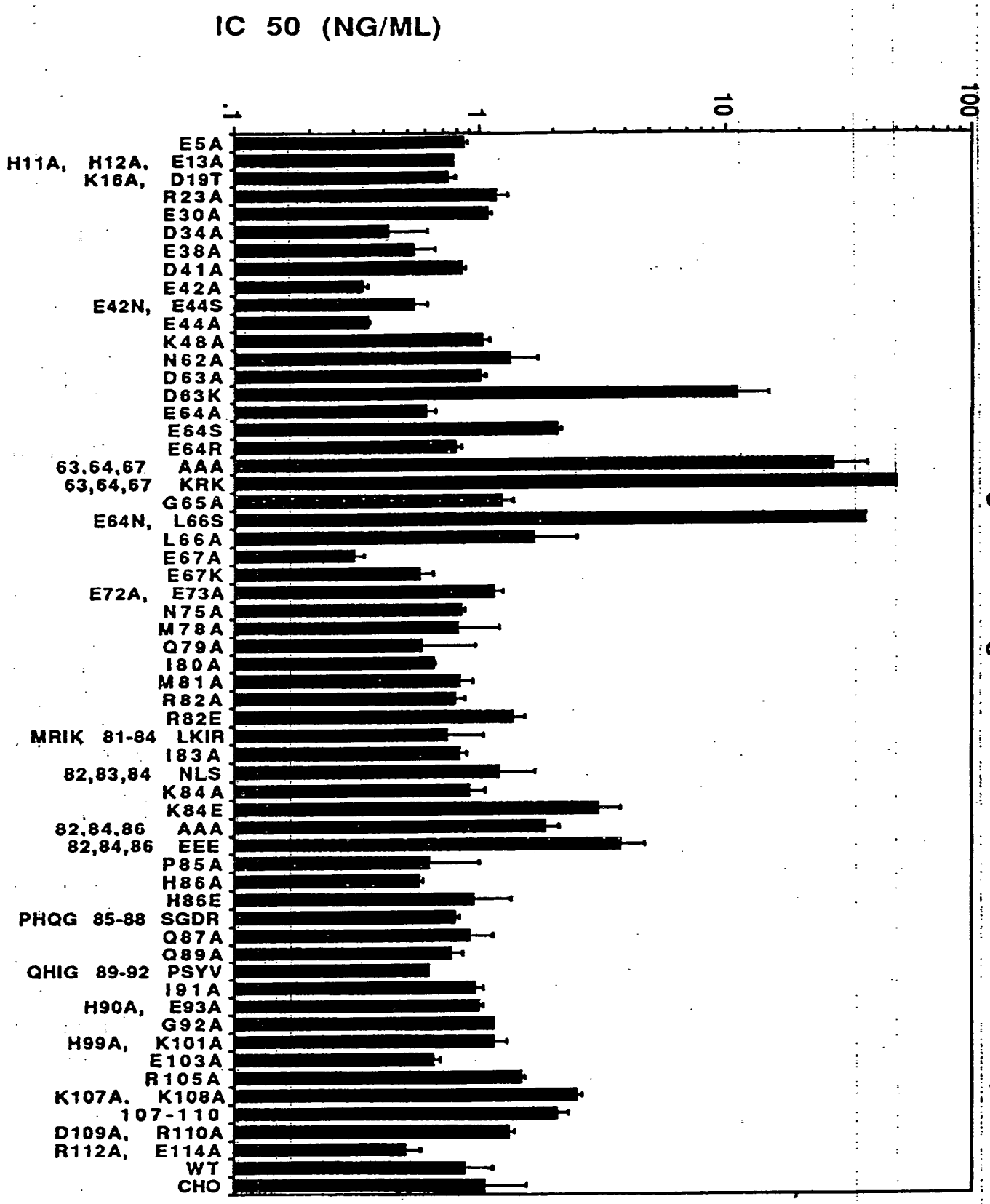

IC 50 (NG/ML)
.1
1
10
100
E5A
H11A, H12A, E13A
K16A, D19T
R23A
E30A
D34A
E38A
D41A
E42A
E42N, E44S
E44A
K48A
N62A
D63A
D63K
E64A
E64S
E64R
63,64,67 AAA
63,64,67 KRK
G65A
E64N, L66S
L66A
E67A
E67K
E72A, E73A
N75A
M78A
Q79A
I80A
M81A
R82A
R82E
MRIK 81-84 LKIR
I83A
82,83,84 NLS
K84A
K84E
82,84,86 AAA
82,84,86 EEE
P85A
H86A
H86E
PHQG 85-88 SGDR
Q87A
Q89A
QHIG 89-92 PSYV
I91A
H90A, E93A
G92A
H99A, K101A
E103A
R105A
K107A, K108A
107-110
D109A, R110A
R112A, E114A
WT
CHO
FLT1-IgG Binding of VEGF Variants
no heparin

FIG. 17

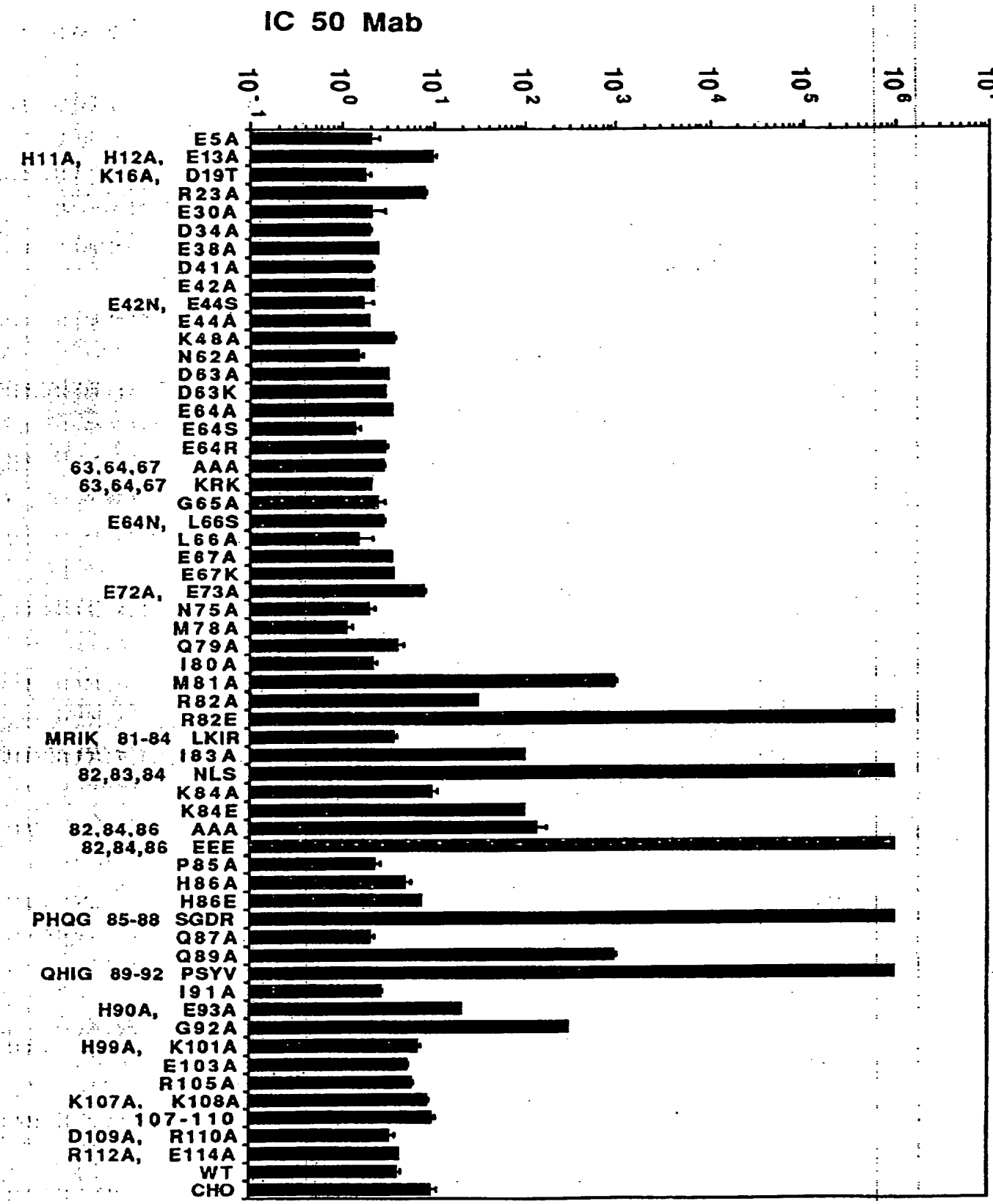

IC 50 Mab
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
10^{7}
E5A
H11A, H12A, E13A
K16A, D19T
R23A
E30A
D34A
E38A
D41A
E42A
E42N, E44S
E44A
K48A
N62A
D63A
D63K
E64A
E64S
E64R
63,64,67 AAA
63,64,67 KRK
G65A
E64N, L66S
L66A
E67A
E67K
E72A, E73A
N75A
M78A
Q79A
I80A
M81A
R82A
R82E
MRIK 81-84 LKIR
I83A
82,83,84 NLS
K84A
K84E
82,84,86 AAA
82,84,86 EEE
P85A
H86A
H86E
PHQG 85-88 SGDR
Q87A
Q89A
QHIG 89-92 PSYV
I91A
H90A, E93A
G92A
H99A, K101A
E103A
R105A
K107A, K108A
107-110
D109A, R110A
R112A, E114A
WT
CHO
A461 Binding of VEGF Variants

FIG. 18

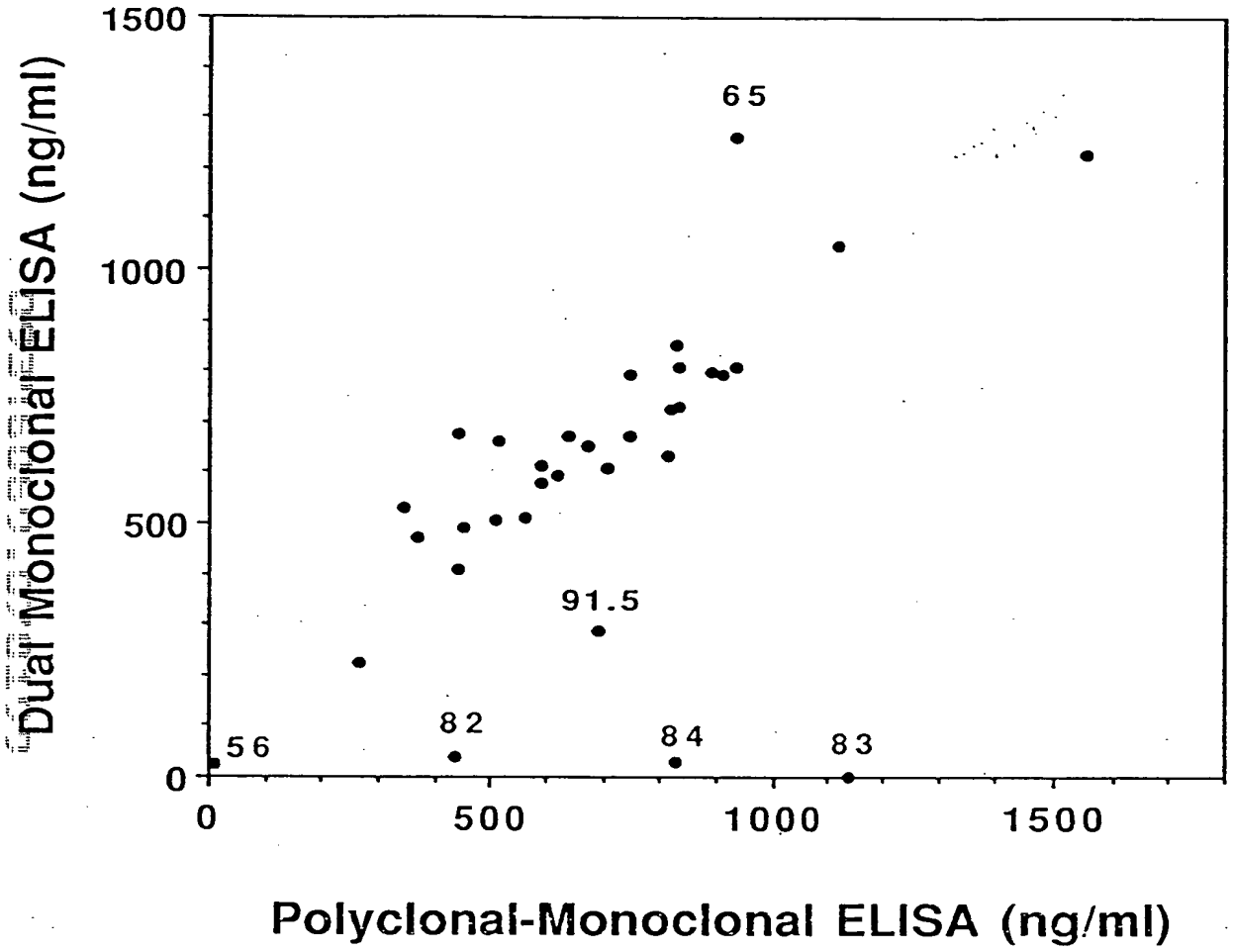

1500
1000
500
0
0
500
1000
1500
Dual Monoclonal ELISA (ng/ml)
Polyclonal-Monoclonal ELISA (ng/ml)
65
91.5
56
82
84
83

FIG. 19

293 VEGF
E64N, L66S
E67A
E72A, E73A
N75A
R82A
R82N, I83L, K84S
K84A
R82A, K84A, H86A
Blank
293 VEGF
H86A
H90A, E93A
H99A, K101A
R103A
R105A
K107A, K108A
KKDR(107-110)AAAA
D109A, R110A
R112A, E114A
293 VEGF
E5A
HHE(11-13)AAA
K16A, D19T
R23A
E30A
D34A
E38A
D41A
CHO VEGF
293 VEGF
E42A
E42N, E44S
E44A
K48A
D63A
E64A
E64S
D63A, E64A, E67A
Blank
107
76
52
37
27
19

FIG. 20